

Amendments to the Claims:

1. (Currently Amended) A method of labeling a molecule exposed on a luminal surface of a cell lining of a perfusible space *in situ* or *in vivo* comprising the following steps:
 - (a) providing a cell membrane impermeable reagent comprising three domains
 - (i) a first domain comprising a chemical moiety capable of covalently and non-specifically binding to a molecule exposed on the luminal surface of a cell lining of a perfusible space *in situ* or *in vivo*,
 - (ii) a second domain comprising a labeling domain, and
 - (iii) a third domain situated between the first and second domains linking the first domain to the second domain by a cleavable chemical moiety, wherein the cleavable chemical moiety will not cleave is not cleavable under *in vivo* conditions but is cleavable under a condition that does not denature the lumen-exposed molecule; and
 - (b) administering the membrane impermeable reagent into the perfusible space in an intact organ or an intact animal to react the cell membrane impermeable reagent with the molecule expressed on the luminal surface of the cell lining of the perfusible space to label a the lumen-exposed molecule; and
 - (c) cleaving the cleavable chemical moiety of the reagent that reacted with the lumen-exposed molecule under the condition that does not denature the lumen-exposed molecule.
2. (Original) The method of claim 1, wherein the lumen-exposed molecule is an organ-specific or a tissue-specific molecule.
3. (Original) The method of claim 1, wherein the perfusible space is a lumen of a vascular vessel and the cell lining the space is an endothelial cell.
4. (Original) The method of claim 3, wherein the vascular vessel is an artery, an arteriole, a vein, or a capillary.
5. (Original) The method of claim 1, wherein the perfusible space is a lumen of a cerebral spinal fluid (CSF) space.

6. (Original) The method of claim 1, wherein the perfusible space is a lumen of a lymphatic vessel and the cell lining the space is an endothelial cell.

7. (Original) The method of claim 1, wherein the perfusible space is a lumen of an endocrine or exocrine duct or pore.

8. (Original) The method of claim 1, wherein the cell lining the perfusible space is an epithelial cell.

9. (Original) The method of claim 1, wherein the organ is, or the tissue is derived from, a heart, a lung, a brain, a liver, a kidney, an endocrine gland, skin, a reproductive organ, a digestive tract organ, or an eye.

10. (Currently Amended) The method of claim 1, wherein the labeling domain of the reagent comprises biotin is selected from the group consisting of an enzyme, biotin, a colorimetric moiety, a fluorescent moiety, a luminescent moiety, a bioluminescent moiety, a radionucleotide and a paramagnetic element.

11.-12. (Cancelled)

13. (Original) The method of claim 1, wherein the cleavable chemical moiety comprises a disulfide group.

14. (Withdrawn) The method of claim 1, wherein the cleavable chemical moiety comprises a periodate-cleavable glycol, a dithionite-cleavable diazobond, a hydroxylamine-cleavable ester or a base-labile sulfone.

15. (Cancelled)

16. (Original) The method of claim 1, wherein administering the cell membrane impermeable reagent into the perfusible space of the intact organ or tissue or the intact animal comprises administration of a buffered, aqueous solution comprising the cell membrane impermeable reagent.

17. (Original) The method of claim 1, wherein the molecule exposed on the luminal surface of the perfusible space and labeled by the cell membrane impermeable reagent is a polypeptide.

18. (Original) The method of claim 1, wherein the molecule exposed on the luminal surface of the perfusible space and labeled by the cell membrane impermeable reagent is a lipid or a carbohydrate.

19. (Currently Amended) A method of isolating a molecule that is exposed on a luminal surface of a perfusible space comprising the following steps:

(a) providing a cell membrane impermeable reagent comprising three domains

(i) a first domain comprising a chemical moiety capable of covalently and non-specifically binding to a molecule expressed on the luminal surface of a cell lining a perfusible space *in situ* or *in vivo*,

(ii) a second domain comprising a binding domain;

(iii) a third domain situated between the first and second domains linking the first domain to the second domain by a cleavable chemical moiety, wherein the cleavable chemical moiety ~~will not cleave is not cleavable~~ but is cleavable under a condition that does not denature the lumen-exposed molecule; and

(b) administering the cell membrane impermeable reagent into the perfusible space in an intact organ or an intact animal to react the cell membrane impermeable reagent with a molecule expressed on the luminal surface of the cell lining of the perfusible space; and

(c) isolating the lumen-exposed molecule that reacted with the reagent under the condition that does not denature the lumen-exposed molecule. ~~a reagent reacted molecule~~.

20. (Original) The method of claim 19, wherein the lumen-exposed molecule is an organ-specific or a tissue-specific molecule.

21. (Currently Amended) The method of claim 20, further comprising the step of comparing the reagent-reacted molecules from different organs or tissues to identify ~~an~~ the organ-specific or

tissue-specific molecule, wherein the organ-specific or tissue-specific molecule is exposed on the luminal surface of the perfusible space of only one of the compared organs or tissues.

22. (Original) The method of claim 19, wherein the perfusible space is a lumen of a vascular vessel and the cell lining the space is an endothelial cell.

23. (Original) The method of claim 22, wherein the vascular vessel is an artery, an arteriole, a vein, or a capillary.

24. (Original) The method of claim 19, wherein the perfusible space is a lumen of a cerebral spinal fluid (CSF) space.

25. (Original) The method of claim 19, wherein the perfusible space is a lumen of a lymphatic vessel and the cell lining the space is an endothelial cell.

26. (Original) The method of claim 19, wherein the perfusible space is a lumen of an endocrine or exocrine duct or pore.

27. (Original) The method of claim 19, wherein the cell lining of the perfusible space is an epithelial cell.

28. (Original) The method of claim 19, wherein the organ is, or the tissue is derived from, a heart, a lung, a brain, a liver, a kidney, an endocrine gland, skin, a reproductive organ, a digestive tract organ, or an eye.

29. (Original) The method of claim 19, wherein the binding domain of the reagent comprises biotin.

30. (Currently Amended) The method of claim 19, wherein the binding domain of the reagent comprises a polypeptide, a nucleic acid, or a peptide nucleic acid, ~~a naturally occurring or a synthetic organic molecule or a chelate~~.

31. (Withdrawn) The method of claim 30, wherein the polypeptide comprises a polyhistidine, a protein A domain, or a FLAG extension.
32. (Original) The method of claim 19, wherein the cleavable chemical moiety comprises a disulfide group.
33. (Withdrawn) The method of claim 19, wherein the cleavable chemical moiety comprises a disulfide group a periodate-cleavable glycol, a dithionite-cleavable diazobond, a hydroxylamine-cleavable ester or a base-labile sulfone.
34. (Withdrawn) The method of claim 19, wherein the cell membrane impermeable reagent further comprises a fourth domain comprising a molecule that facilitates detection of the reagent.
35. (Cancelled)
36. (Original) The method of claim 19, wherein administering the cell membrane impermeable reagent into the perfusible space of the intact organ or tissue or the intact animal comprises administration of a buffered, aqueous solution comprising the cell membrane impermeable reagent.
37. (Original) The method of claim 19, wherein the molecule exposed on the luminal surface of the perfusible space and isolated by the cell membrane impermeable reagent is a polypeptide.
38. (Original) The method of claim 19, wherein the molecule exposed on the luminal surface of the perfusible space and isolated by the cell membrane impermeable reagent is a lipid or a carbohydrate.
39. (Original) The method of claim 19, wherein two separate cell membrane impermeable reagents are co-administered.
40. (Original) The method of claim 19, wherein the reagent-reacted molecule is isolated by

- (a) contacting a cell or a membrane isolate or a cell or a tissue homogenate or an extract derived from the reagent-reacted organ or animal with a ligand having affinity for the binding domain of the cell membrane impermeable reagent; and
- (b) removing a non-bound molecule from the ligand-bound molecules.

41. (Original) The method of claim 40, wherein the ligand is immobilized.

42. (Original) The method of claim 41, wherein the ligand is immobilized on a bead.

43. (Original) The method of claim 40, wherein the ~~binding domain~~ ligand is an avidin or a strepavidin molecule.

44. (Original) The method of claim 40, wherein the reagent-reacted molecule is further isolated by removing substantially all of the non-bound molecule from the ligand-bound molecules.

45. (Original) The method of claim 40, wherein the non-bound molecule is removed by washing.

46. (Currently Amended) The method of claim 40, wherein the reagent-reacted molecule is further isolated by ~~cleavage of~~ cleaving the cleavable chemical moiety of the cell membrane impermeable reagent under a condition that does not denature the lumen-exposed molecule and does not dissociate the ligand from the binding domain after removing a non-bound molecule.

47.-48. (Cancelled)

49. (Currently Amended) The method of claim 46, wherein the reagent-reacted lumen-exposed molecule is further isolated by elution from the binding domain and the ligand.

50. (Cancelled)

51. (Currently Amended) A method of isolating an organ-specific or tissue-specific molecule that is exposed on a luminal surface of an arteriole, a capillary or a vein comprising the following steps:

- (a) providing a cell membrane impermeable reagent comprising three domains
 - (i) a first domain comprising an active moiety capable of covalently and non-specifically binding to a molecule expressed on the luminal surface of a cell lining a perfusible space *in situ* or *in vivo*,
 - (ii) a second domain comprising a biotin binding domain, and
 - (iii) a third domain comprising a disulfide moiety situated between the first and second domains linking the first domain to the second domain; and
- (b) administering the cell membrane impermeable reagent into a lumen of an artery, a arteriole, a capillary or a vein in an intact organ or an intact animal to react the cell membrane impermeable reagent with a molecule expressed on the luminal surface; and
- (d) isolating the reagent-reacted molecule by contacting the reagent reacted molecule with an immobilized avidin or streptavidin molecule; and removing substantially all of the non-immobilized molecules.

52.-54. (Cancelled)

55. (Currently Amended) A method of labeling a molecule exposed on a luminal surface of a perfusible space *in situ* or *in vivo* comprising the following steps:

- (a) providing a cell membrane impermeable reagent comprising three domains:
 - (i) a first domain comprising a chemical moiety capable of covalently and non-specifically binding to a molecule exposed on the luminal surface of a cell lining a perfusible space *in situ* or *in vivo*,
 - (ii) a second domain comprising a labeling domain, and
 - (iii) a third domain situated between the first and second domains linking the first domain to the second domain by a cleavable chemical moiety, wherein the cleavable chemical moiety will not cleave under *in vivo* conditions, and further wherein the cell membrane impermeable reagent is sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate; and

- (b) administering the membrane impermeable reagent into the perfusible space in an intact organ or an intact animal to react the cell membrane impermeable reagent with the molecule expressed on the luminal surface of the cell lining the perfusible space to label a lumen-exposed molecule; and
- (c) cleaving the cleavable chemical moiety under a condition that does not denature the lumen-exposed molecule.

56. (New) The method of claim 10, wherein the enzyme is selected from the group consisting of horseradish peroxidase, alkaline phosphatase, β -galactosidase, and acetylcholinesterase.

57. (New) The method of claim 11, wherein the bioluminescent moiety is selected from the group consisting of luciferase, luciferin, and aequorin.

58. (New) The method of claim 10, wherein the radionucleotide is selected from the group consisting of H-3, S-35, I-125, I-131, P-32, Y-90, Re-188, At-211, and Bi-212.

59. (New) The method of claim 10, wherein the paramagnetic moiety is selected from the group consisting of Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu.

60. (New) The method of claim 10, wherein the fluorescent moiety is selected from the group consisting of umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, and phycoerythrin.

61. (New) The method of claim 51, further comprising the step of cleaving the cleavable chemical moiety of the cell membrane impermeable reagent under a condition that does not dissociate said immobilized avidin or streptavidin molecule from said biotin binding domain.

REMARKS

Claims 14, 31, 33-34 are withdrawn. Claims 11-12, 15, 35, 47-48, 50, 52-54 are cancelled. Claims 1, 10, 19, 21, 31, 46, 51 and 55 are amended. Claims 56-61 are added. Claims 1-10, 13-14, 16-34, 36-46, 49, 51, 55-61 are now pending. Reconsideration is respectfully requested in view of the following remarks.

I. Claim Objections

Claims 1, 11, 19, 30, 51, and 52 were objected to on the basis that they recite species that were not specifically elected.

A restriction requirement was made by a previous Examiner on July 5, 2001, requesting Applicants to elect between two species. The first specie (group I) covered a cell membrane impermeable reagent wherein the second domain of the reagent is a labeling domain. The second specie (group II) covered a cell membrane impermeable reagent wherein the second domain of the reagent is a binding domain.

During the telephone interview with the Examiner, it was agreed that the labeling domain and detecting domains are not independent and distinct species of invention and that the restriction requirement should be withdrawn. Therefore, Applicants respectfully request that the above claim objections be withdrawn.

II. Claim Rejections Under 35 U.S.C. 112, First Paragraph:

The Examiner rejected claims 52-54 under 35 U.S.C. 112, First Paragraph. As a result of Applicants' cancellation of claims 52-54, the above rejection is moot. Applicants respectfully request that the Examiner withdraw the above rejection.

III. Claim Rejections Under 35 U.S.C. 102(b):

The Examiner rejected claims 52-53 under 35 U.S.C. 102(b). As a result of Applicants' cancellation of claims 52 and 53, the above rejection is moot. Applicants respectfully request that the Examiner withdraw the above rejection.

IV. Claim Rejections Under 35 U.S.C. 103(a):

The Examiner rejected claims 1-11, 13, 16-30, 32, 36-51 and 55 under 35 U.S.C. 103(a) as being unpatentable over De La Fuente *et al.*, Hastie *et al.*, Rothschild *et al.* and Pierce Catalog

& Handbook, 1994-1995. In particular, the Examiner stated that the above references render the claimed invention obvious by providing a reasonable expectation of success. Applicants respectfully traverse the Examiner's rejection under 35 U.S.C. 103(a) for the following reasons.

Independent claims 1 and 19 as amended are directed to a method of labeling and a method of isolating a lumen-exposed molecule, respectively. Specifically, the claimed methods include a step of administering a cell membrane impermeable reagent having a cleavable chemical moiety that "is not cleavable under *in vivo* conditions but is cleavable under a condition that does not denature the lumen exposed molecules." Support for the amended language appears in the specification, for example, on page 8, lines 3-4 stating that "[i]n one embodiment, the conditions for cleaving the cleavable chemical moiety do not denature the reacted and isolated molecule" and on page 5, lines 8-11, which provide that the cleavable chemical moiety can be cleaved under relatively mild conditions such as "non-denaturing conditions."

None of the references cited by the Examiner teaches or motivates the claimed invention. In particular, De La Fuente *et al.* and Hastie *et al.* disclose the use of sulfosuccinimidyl 6-biotinamido hexanoate (NHS-LC-Biotin) for the identification and isolation of lumen-exposed molecules but fail to teach or motivate the use of an impermeable cell-membrane reagent that is **cleavable**. Rothschild *et al.* provides allegedly a general description of heterobifunctional crosslinkers for use in detection and isolation of biomolecules but fails to teach or motivate the administration of reagents into a perfusible space. In addition, Pierce Catalog & Handbook teaches the use of cleavable NHS-SS-biotin for isolating proteins from a mixture of proteins but fails to teach or motivate the administration of any reagents into a perfusible space.

None of the above references teaches or suggests, independently or in combination with another reference, the claimed method employing a cell membrane impermeable reagent with the following three structural domains:

- First domain: a chemical moiety domain that can covalently and non-specifically bind to a molecule exposed on the luminal surface of a cell lining of a perfusible space;
- Second domain: a labeling domain or a binding domain; and
- Third domain: a cleavable chemical moiety that is not cleavable under *in vivo* conditions but is **cleavable under a condition that will not denature the lumen-exposed molecule**, and/or optionally under **a condition that will not dissociate a complex** formed between the binding domain (e.g., biotin) and its ligand (e.g., avidin).

Independent claims 1, 19, and 51 utilize the above reagent and, therefore, are not obvious in light of the above references.

Furthermore, none of the above references teaches or suggests a method of labeling a molecule exposed on a luminal surface of a perfusible space *in situ* or *in vivo*, by administering to a perfusible space sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate as is described in claim 55. Therefore, claim 55 is not obvious in light of the above references either.

As none of the above references, independently or in combination, teaches or suggests the claimed invention, a *prima facie* case of obviousness has not been established. Therefore, Applicants respectfully requests that the rejection under 35 U.S.C. §103(a) be withdrawn.

CONCLUSION

In light of the remarks set forth above, Applicants believe that they are entitled to a letters patent. Applicants respectfully solicit the Examiner to expedite the prosecution of this patent application to issuance. Should the Examiner have any question, the Examiner is encouraged to telephone the undersigned.

Respectfully submitted,

Date: 8/26/03 By:

Maya Skubatch
Maya Skubatch
Registration No. 52,505

WILSON SONSINI GOODRICH & ROSATI
650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 849-3330
Client No. 021971